Published in final edited form as:

Organometallics. 2011 July 18; 30(17): 4598–4606. doi:10.1021/om200366r.

Organometallic Pyridyl-naphthalimide Complexes as Protein Kinase Inhibitors

Sebastian Blanck[†], Thomas Cruchter[†], Adina Vultur[‡], Radostan Riedel[†], Klaus Harms[†], Meenhard Herlyn[‡], and Eric Meggers^{*,†}

[†]Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35043 Marburg, Germany

[‡]The Wistar Institute, 3601 Spruce Street, Philadelphia, PA, 19104, USA

Abstract

A new metal-containing scaffold for the design of protein kinase inhibitors is introduced. Key feature is a 3-(2-pyridyl)-1,8-naphthalimide “pharmacophore chelate ligand” which is designed to form two hydrogen bonds with the hinge region of the ATP-binding site and is at the same time capable of serving as a stable bidentate ligand through C-H-activation at the 4-position of the electron-deficient naphthalene moiety. This C-H-activation leads to a reduced demand for coordinating heteroatoms and thus sets the basis for a very efficient three-step synthesis starting from 1,8-naphthalic anhydride. The versatility of this ligand is demonstrated with the discovery of a ruthenium complex that functions as a nanomolar inhibitor for myosin light-chain kinase (MYLK or MLCK).

Introduction

The last two decades have witnessed a steadily growing interest for organometallic compounds in medicinal chemistry and chemical biology.¹ Unique properties of metal complexes such as structural diversity, adjustable ligand exchange kinetics, fine-tuned redox activities, photoreactivity, the availability of radioisotopes, and distinct spectroscopic signatures, make them highly versatile scaffolds for the modulation, sensing, and imaging of biological processes.²

Several years ago we established a research program exploring substitutionally inert metal-containing compounds as structural scaffolds for the design of enzyme inhibitors and we demonstrated that ruthenium(II),³ platinum(II),⁴ osmium(II),⁵ and iridium(III)⁶ complexes, for example, can serve as highly potent and selective inhibitors of protein kinases and lipid kinases.⁷ We believe that especially octahedral coordination modes offer new opportunities to design globular and well-defined molecular shapes that can fill protein pockets such as enzyme active sites in unique ways.⁸

Our previous design was entirely based on the staurosporine-inspired metallopyridocarbazole scaffold shown in Figure 1 in which the maleimide moiety forms two key hydrogen bonds with the hinge region of the ATP-binding site, the pyridocarbazole heterocycle occupies the hydrophobic adenine binding cleft, and the remaining globular

*meggers@chemie.uni-marburg.de.

Supporting Information Available: Screening data of compound **7** againsts a panel of 442 protein kinases, stability test with **7**, investigation of the binding mode of the ambidentate NCS and NCSe ligands in the complexes **8** and **9**, synthesis and analytical data of the complex **7Me**, and complete ref. 17, 18, and 22. This material is available free of charge via the Internet at <http://pubs.acs.org>.

space-demanding octahedral or half-sandwich coordination sphere undergoes interactions in the region of the ribose-triphosphate binding site.⁹ In this design strategy, the pyridocarbazole heterocycle – called “pharmacophore chelate ligand” – is rationally designed to interact with the hinge-region of the ATP-binding site of protein kinases whereas the remaining coordination sphere can then be established through combinatorial chemistry, structure-activity relationships, structure-based design, and combinations thereof. Although this approach turned out to be highly successful, we recently became interested in designing new pharmacophore chelate ligands for metal-containing protein kinase inhibitors for two reasons: First, the synthesis of the pyridocarbazole ligand is lengthy and includes one photochemical step, thus complicating derivatizations in the course of structure-activity-relationships and inhibitor scale-up reactions for *in vivo* studies.¹⁰ Second, we envisioned that placing the metal center at a different position within the ATP-binding site might allow us to discover metal-containing inhibitors with very different binding profiles. In this respect it is noteworthy that protein kinases are amongst the largest enzyme families with more than 500 putative protein kinase genes¹¹ and we estimate that our previous pyridocarbazole metal complex scaffold is probably only suitable for a subset of them. Here we now introduce 3-(2-pyridyl)-1,8-naphthalimide (**1a**, Scheme 2) as such a novel pharmacophore ligand. It can be synthesized in just three steps from readily available 1,8-naphthalic anhydride, coordinates to ruthenium in a bidentate fashion by activating a C-H-bond within the naphthalimide moiety, and as a proof-of-principle we report an organoruthenium complex which serves as a selective and nanomolar protein kinase inhibitor.

Results and Discussion

Synthesis of the 3-(2-pyridyl)-1,8-naphthalimide ligand

The synthesis of the 3-(2-pyridyl)-1,8-naphthalimide ligand **1a** (R = H) and its benzylated derivative **1b** (R = Bn), which merely served as a crystallization handle, is outlined in Scheme 1. Accordingly, a bromination of 1,8-naphthalic anhydride **2** in concentrated sulfuric acid using silver(I) sulfate as catalyst afforded exclusively 3-bromo-1,8-naphthalic anhydride **3** (91%), which was further converted to the free imide **4a** (93%) or the benzyl-protected imide **4b** (71%) by treatment with concentrated ammonia or benzylamine, respectively. A subsequent Pd-mediated *Stille* cross coupling with 2-(trimethylstannyl)pyridine provided the final ligands **1a** (67%) and **1b** (84%) in overall three steps and total yields of 57% and 54%, respectively.

Synthesis of half-sandwich compounds

To investigate the coordination properties of the new pyridynaphthalimide ligands we started with the synthesis of ruthenium half-sandwich complexes. For example, the reaction of the ligands **1a** and **1b** with $[\text{Ru}(\eta^5\text{-C}_5\text{H}_5)(\text{CO})(\text{MeCN})_2]\text{PF}_6$ ¹² in the presence of Et_3N at 60 °C provided the complexes **5a** (51%) and **5b** (63%) which could be purified by standard silica gel flash chromatography (Scheme 2). As anticipated, the C-H-activation occurred solely at the 4-position of the naphthalimide moiety while no C-H-activation at the sterically more hindered 2-position was observed. A crystal structure of the complex **5b** is displayed in Figure 2 and confirms the bidentate binding of the ruthenium to the pyridine nitrogen (Ru-N = 2.10 Å) and the C-4 atom of the naphthalene moiety (Ru-C = 2.23 Å). It is worth noting that the facile C-H activation and robustness of the formed Ru-C bond can be attributed to the highly electron-deficient nature of the naphthalene moiety, being functionalized with electron-withdrawing pyridyl and maleimide substituents.¹³

Synthesis of octahedral complexes

In order to evaluate the synthesis of octahedral ruthenium complexes, ligand **1a** was reacted with $[\text{Ru}(\text{MeCN})_3([9]\text{aneS}_3)](\text{CF}_3\text{SO}_3)_2$,¹⁴ $[9]\text{aneS}_3$ = 1,4,7-trithiacyclononane, and

triethylamine in DMF at 60 °C to obtain the mono-acetonitrile complex **6** in 77% yield (Scheme 3). This complex offers a quick access to different octahedral complexes by exchanging the remaining semilabile acetonitrile against other monodentate ligands. For example, heating the acetonitrile complex **6** under an atmosphere of CO for 3 h provided the CO complex **7** in 87% yield. The synthesis of the thiocyanate complex **8** and the selenocyanate complex **9** were accomplished by reacting complex **6** with NaSCN (23% yield) and KSeCN (30% yield), respectively. All three complexes were purified by regular silica gel flash chromatography. Complex **7** was exemplarily tested for its stability under biologically relevant conditions and was found to be stable in aqueous solution under air in the presence of millimolar concentrations of 2-mercaptoethanol for several days without any sign of decomposition as determined by ¹H-NMR spectroscopy.

A crystal structure of the complex **7** displayed in Figure 3 reveals that in contrast to the half-sandwich complex **5b**, the pyridylnaphthalimide ligand in the octahedral complex is twisted, which is apparently due to a steric repulsion between the C-H-group at the 5-position of the naphthalimide moiety and the sterically demanding 1,4,7-trithiacyclononane ligand. This steric repulsion also results in a significant distortion of the entire octahedral ruthenium coordination geometry. For example, the CO ligand is not oriented perpendicular to the plane of the naphthalimide moiety but instead bent backwards. Interestingly, the crystal structure furthermore demonstrates the desired ability of the imide moiety to form hydrogen bonds.

Synthesis of a reactive “precursor complex”

For future combinatorial chemistry it would be desirable to have access to a complex in which the ruthenium is coordinated to the pyridylnaphthalimide pharmacophore ligand in addition to labile monodentate coordinating ligands that can be replaced in a combinatorial fashion against other mono-, bi-, tri-, or even tetradentate ligands, thus providing a rapid access to a diversity of novel structures just by straightforward ligand substitution chemistry.¹⁵ In fact, the reaction of pyridylnaphthalimide **1a** with [Ru(η⁶-C₆H₆)Cl₂]₂ in the presence of K₂CO₃ and KPF₆ in refluxing acetonitrile afforded in one step the tetraacetonitrile complex **10** in a yield of 40% (Scheme 3).¹⁶ A crystal structure of complex **10** is shown in Figure 4. Despite the apparent lability of the four acetonitrile ligands, complex **10** is surprisingly robust and can be purified by regular silica gel flash chromatography. As expected, the acetonitrile ligands of **10** are prone to substitution under elevated temperature. For example, the reaction of **10** with 1,4,7-trithiacyclononane in DMF provided in a good yield of 73% the complex **6** in which three acetonitrile ligands are replaced by 1,4,7-trithiacyclononane.

Protein kinase inhibition

To quickly gain insight into the protein kinase inhibition properties of pyridylnaphthalimide metal complexes, we tested complex **7** as a representative member of this class of compounds for its protein kinase binding affinity profile against the majority of the human protein kinases encoded in the human genome (human kinome).¹¹ This was accomplished by using an active-site-directed competition binding assay with 442 different protein kinases (KINOMEscan, DiscoveRx) which provides primary data (%ctrl = percent of control: 0% = highest affinity, 100% = no affinity) that correlate with binding constants (*K_d*).^{17,18} Interestingly, out of the tested 442 enzymes, eight protein kinases, namely CLK2, DMPK, HIPK2, MAP3K8, MYLK, PKCθ, RSK2, and RSK4 were identified as the main hits with %ctrl values below 0.1%. The human kinase dendrogram shown in Figure 5 reveals that these eight kinases are distributed among four protein kinase families. We selected myosin light-chain kinase (MYLK or MLCK) for further studies because MYLK plays a very important role in the smooth muscle contraction.¹⁹ Upon stimulation by agonists, the

intracellular concentration of Ca^{2+} of smooth muscle is elevated, causing Ca^{2+} to bind to calmodulin. The complex of Ca^{2+} and calmodulin then activates MYLK. We determined complex **7** to be a nanomolar inhibitor of MYLK with an IC_{50} value (concentration of inhibitor at which the enzyme activity is reduced to 50%) of 75 ± 20 nM ($100 \mu\text{M}$ ATP), which according to the Cheng-Prusoff equation ($K_m(\text{ATP}) = 50 \mu\text{M}$) would reflect an inhibition constant (K_d) of approximately 25 nM.^{20,21} Surprisingly, the pyridyl-naphthalimide ligand **1a** itself does not show any sign of MYLK inhibition even at a concentration of 1 mM. Thus, the Ru(1,4,7-trithiacyclononane)(CO)-fragment increases the binding affinity by more than 4 orders of magnitude. To evaluate if complex **7** binds as designed to the hinge region of the ATP binding site, we synthesized a derivative of **7** in which the imide hydrogen is replaced by a methyl group (**7Me**). As shown in Figure 6, this methylation dramatically reduces the activity by almost two orders of magnitude ($\text{IC}_{50} = 5.2 \pm 1.0 \mu\text{M}$), consistent with the expectation that the imide hydrogen forms a hydrogen bond with a carbonyl oxygen of the hinge region as indicated in Figure 1. It is also worth noting that neither the half-sandwich complex **5a** ($\text{IC}_{50} = 83 \pm 36 \mu\text{M}$) nor the thiocyanate **8** (IC_{50} of $3.5 \pm 0.9 \mu\text{M}$) or selenocyanate **9** ($\text{IC}_{50} = 4.2 \pm 1.3 \mu\text{M}$) display any significant affinity for MYLK, indicating that it is the bent position of the coordinated CO that is crucial for MYLK binding, most likely through a specific interaction with a region of the flexible glycine-rich loop.

Activities in human cells

Finally, because of the recent clinical success of kinase inhibitors for the treatment of metastatic melanoma,^{22,23} we investigated the complexes **5a** and **7** as representative members of the new class of metallo-naphthalimides in an in vitro MTS proliferation assay²⁴ with two human-derived melanoma cell lines (451Lu and WM3918), as well as human melanocytes (FOM102010) and human fibroblast cells (FF2508) as controls (Figure 7). Unfortunately, even at a concentration as high as $20 \mu\text{M}$, complex **7** did not show any significant effects on the melanoma or normal human cell lines (Figure 7a). It can be assumed that this is due to the cationic nature of complex **7** which may prevent an efficient cellular uptake. In fact, in contrast to complex **7**, the neutral complex **5a** inhibited the proliferation of the melanoma cell lines 451Lu and WM3918 in a dose dependent fashion to 53% and 40% at $20 \mu\text{M}$, respectively, while fibroblasts were only slightly affected and melanocytes not at all (Figure 7b). Thus, due to this promising therapeutic window, complex **5a** and derivatives thereof should be further evaluated for their anti-melanoma activities.

Conclusions

In summary, we here introduced 3-(2-pyridyl)-1,8-naphthalimide as a novel pharmacophore chelate ligand for metal-based protein kinase inhibitors and demonstrated its versatility by presenting a ruthenium complex as nanomolar inhibitor of myosin light-chain kinase. 3-(2-Pyridyl)-1,8-naphthalimide is designed to interact with the hinge region of the ATP-binding site by forming two hydrogen bonds with the imide moiety. A key feature is its capability to serve as a stable bidentate ligand through C-H-activation at the 4-position of the electron-deficient naphthalene moiety which simplifies the structure by reducing the number of required heteroatoms for transition metal binding.

Experimental Section

General methods

All reactions were carried out using oven-dried glassware and conducted under a positive pressure of nitrogen unless otherwise specified. 1,8-Naphthalic anhydride, silver(I)sulfate and other chemicals were used as received from standard suppliers unless otherwise

specified. 3-Bromo-1,8-naphthalic anhydride,²⁵ 2-(trimethylstannyl)pyridine,²⁶ tetrakis(triphenylphosphine)-palladium(0),²⁷ $[\text{Ru}(\eta^5\text{-C}_5\text{H}_5)(\text{CO})(\text{MeCN})_2]\text{PF}_6$,¹² $[\text{Ru}(\text{MeCN})_3][9\text{-aneS}_3](\text{CF}_3\text{SO}_3)_2$,¹⁴ and $[\text{RuCl}_2(\eta^6\text{-C}_6\text{H}_6)]_2$ ²⁸ were prepared according to literature procedures. All solvents were distilled prior to use. Acetonitrile and DMF were dried by common methods and freshly distilled prior to use. NMR spectra were recorded on an Avance 300 (300 MHz), DRX-400 (400 MHz), Avance 500 (500 MHz), or DRX-500 (500 MHz) spectrometer. Infrared spectra were recorded on a Bruker Alpha FTIR. High resolution mass spectra were obtained with a Finnigan LTQ-FT instrument using either APCI or ESI. Complex **7Me** was synthesized in analogy to **7** (see Supporting Information).

3-Bromo-1,8-naphthalimide (**4a**)

3-Bromo-1,8-naphthalic anhydride (**3**) (5.07 g, 18.3 mmol) was added to 255 mL of concentrated ammonia (28%) and heated to 70 °C for 90 min. The solution was cooled to ambient temperature, the resulting precipitate was filtered off, washed with ethanol twice, and dried in vacuum to provide 3-bromo-1,8-naphthalimide (**4a**) as an off-white solid (4.71 g, 93%). ¹H-NMR (300 MHz, DMSO-*d*₆): δ (ppm) 11.82 (br s, 1H), 8.70 (d, *J* = 2.1 Hz, 1H), 8.34–8.43 (m, 3H), 7.87 (t, *J* = 7.8 Hz, 1H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ (ppm) 163.7, 162.9, 135.7, 133.3, 132.9, 131.7, 130.2, 128.2, 127.2, 124.5, 122.7, 119.9. IR (film): ν (cm⁻¹) 3180, 3067, 2827, 1693, 1617, 1589, 1408, 1326, 1262, 883, 821, 783, 745, 726, 515, 477, 429. HRMS calcd for C₁₂H₇BrNO₂ (M+H)⁺ 277.9634, found 277.9624.

3-(2-Pyridyl)-1,8-naphthalimide (**1a**)

3-Bromo-1,8-naphthalimide (**3**) (0.95 g, 3.44 mmol) and 2-(trimethylstannyl)pyridine (0.84 g, 3.47 mmol) were dissolved in 15 mL *m*-xylene and the reaction mixture was purged with nitrogen for 15 min. Tetrakis(triphenylphosphine)palladium(0) (197 mg, 0.17 mmol) was added and the mixture heated to reflux for 22 h. After cooling to ambient temperature the solvent was removed, the crude product adsorbed onto silica gel, and subjected to silica gel chromatography with CH₂Cl₂-MeOH (75:1). The combined product eluents were dried *in vacuo* and 3-(2-pyridyl)-1,8-naphthalimide (**1a**) was obtained as pale yellow solid (0.63 g, 67%). ¹H-NMR (300 MHz, DMSO-*d*₆): δ (ppm) 11.77 (s, 1H), 9.11–9.13 (m, 2H), 8.76–8.78 (m, 1H), 8.52 (dd, *J* = 8.1, 0.9 Hz, 1H), 8.42 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.99 (td, *J* = 7.8, 1.8 Hz, 1H), 7.87 (dd, *J* = 8.1, 7.4 Hz, 1H), 7.47 (ddd, *J* = 7.5, 4.8, 0.9 Hz, 1H). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ (ppm) 164.0, 154.2, 149.8, 137.6, 137.0, 134.9, 132.0, 131.4, 130.2, 128.7, 127.9, 127.5, 123.5, 123.1, 122.5, 120.8. IR (film): ν (cm⁻¹) 3174, 3064, 2846, 1672, 1595, 1586, 1414, 1381, 1360, 1337, 1259, 1203, 843, 833, 780, 748, 737, 513, 500, 475, 439, 401. HRMS calcd for C₁₇H₁₀N₂O₂Na (M+Na)⁺ 297.0634, found 297.0633.

N-Benzyl-3-bromo-1,8-naphthalimide (**4b**)

3-Bromo-1,8-naphthalic anhydride (**3**) (3.00 g, 10.8 mmol) was suspended in 75 mL glacial acetic acid, benzylamine (1.78 mL, 16.2 mmol) was added, and the reaction mixture was heated to reflux for 18 h. The reaction mixture was carefully poured into ice-cold water, the resulting beige precipitate was filtered off, and washed with water. The crude product was dissolved in CH₂Cl₂, adsorbed onto silica gel, and subjected to silica gel chromatography with CH₂Cl₂-hexane 3:2. The combined product eluents were dried *in vacuo* to provide *N*-benzyl-3-bromo-1,8-naphthalimide (**4b**) as an off-white solid (2.82 g, 71%). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.66 (d, *J* = 2.1 Hz, 1H), 8.59 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.34 (d, *J* = 2.1 Hz, 1H), 8.01 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.76 (dd, *J* = 8.4, 7.5 Hz, 1H), 7.53–7.56 (m, 2H), 7.24–7.34 (m, 3H), 5.37 (s, 2H). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 163.6, 163.0, 137.0, 135.5, 134.1, 132.82, 132.75, 131.5, 129.1, 128.5, 128.0, 127.6, 126.5, 124.1, 122.8, 121.1, 43.7. IR (film): ν (cm⁻¹) 3065, 3033, 1702, 1662, 1621, 1587, 1432, 1407, 1358,

1322, 1232, 1177, 1154, 1074, 827, 732, 701. HRMS calcd for $C_{19}H_{13}BrNO_2$ ($M+H$)⁺ 368.0106, found 368.0102.

***N*-Benzyl-3-(2-pyridyl)-1,8-naphthalimide (1b)**

N-Benzyl-3-bromo-1,8-naphthalimide (**4b**) (417 mg, 1.14 mmol) and 2-(trimethylstannyl)pyridine (250 mg, 1.04 mmol) were dissolved in 25 mL *m*-xylene and the reaction mixture was purged with nitrogen for 15 min. Tetrakis(triphenylphosphine) palladium(0) (120 mg, 0.10 mmol) was added and the mixture heated to reflux for 22 h. After cooling to ambient temperature the solvent was removed, the crude product adsorbed onto silica gel, and subjected to silica gel chromatography with CH_2Cl_2 -MeOH 75:1 to 35:1. The combined product eluents were dried *in vacuo* and *N*-benzyl-3-(2-pyridyl)-1,8-naphthalimide (**1b**) was obtained as pale yellow solid (381 mg, 99%). ¹H-NMR (300 MHz, $CDCl_3$): δ (ppm) 9.17 (d, J = 1.8 Hz, 1H), 8.95 (d, J = 1.8 Hz, 1H), 8.76–8.78 (m, 1H), 8.59 (dd, J = 7.2, 1.2 Hz, 1H), 8.28 (dd, J = 8.1, 0.9 Hz, 1H), 7.96 (dt, J = 8.1, 0.9 Hz, 1H), 7.85 (td, J = 7.5, 1.8 Hz, 1H), 7.75 (dd, J = 8.1, 7.5 Hz, 1H), 7.56–7.59 (m, 2H), 7.24–7.36 (m, 4H), 5.41 (s, 2H). ¹³C-NMR (75 MHz, $CDCl_3$): δ (ppm) 164.2, 164.1, 155.2, 150.1, 138.0, 137.3, 137.2, 134.8, 132.1, 131.9, 131.7, 129.8, 129.0, 128.4, 128.2, 127.5, 127.4, 123.1, 122.6, 120.8, 43.6. IR (film): ν (cm^{-1}) 3062, 1701, 1662, 1626, 1591, 1476, 1420, 1331, 1200, 1180, 782, 736, 701. HRMS calcd for $C_{24}H_{17}N_2O_2$ ($M+H$)⁺ 365.1285, found 365.1281.

Complex 5a

3-(2-Pyridyl)-1,8-naphthalimide (**4a**) (30 mg, 108 μ mol), and $[Ru(\eta^5-C_5H_5)(CO)(MeCN)_2]PF_6$ (71 mg, 165 μ mol) were dissolved in 5 mL DMF and the reaction mixture was purged with nitrogen for 10 min. Following the addition of triethylamine (35 μ L, 249 μ mol), the mixture was heated to 60 °C for 18 h. The reaction mixture was cooled to ambient temperature, diluted with 50 mL CH_2Cl_2 , and washed with saturated NH_4Cl solution. The aqueous phase was washed with 2 $\times$ 25 mL CH_2Cl_2 , the combined organic phases dried using $MgSO_4$, and the crude product subjected to silica gel chromatography with CH_2Cl_2 -MeOH 40:1. The combined product eluents were dried *in vacuo* and the final complex **5a** was obtained as yellow solid (27 mg, 51%). ¹H-NMR (300 MHz, $DMSO-d_6$): δ (ppm) 11.56 (s, 1H), 9.11 (d, J = 5.7 Hz, 1H), 8.76 (s, 1H), 8.53 (d, J = 8.4 Hz, 1H), 8.44–8.47 (m, 2H), 8.01 (t, J = 7.5 Hz, 1H), 7.80 (t, J = 7.8 Hz, 1H), 7.23 (t, J = 6.6 Hz, 1H), 5.29 (s, 5H). ¹³C-NMR (75 MHz, $DMSO-d_6$): δ (ppm) 201.0, 200.8, 165.9, 164.8, 164.1, 157.7, 144.7, 143.9, 142.8, 142.2, 137.9, 130.3, 125.7, 123.3, 122.7, 121.6, 120.4, 117.3, 85.1. IR (film): ν (cm^{-1}) 2922, 2851, 1934, 1670, 1577, 1557, 1471, 1381, 1318, 1257, 1244, 999, 965, 791, 777, 741. HRMS calcd for $C_{23}H_{15}N_2O_3Ru$ ($M+H$)⁺ 469.0121, found 469.0123.

Complex 5b

N-Benzyl-3-(2-pyridyl)-1,8-naphthalimide (**4b**) (16 mg, 44 μ mol) and $[Ru(\eta^6-C_5H_5)(CO)(MeCN)_2]PF_6$ (28 mg, 66 μ mol) were dissolved in 4 mL DMF and the reaction mixture was purged with nitrogen for 10 min. Following the addition of triethylamine (7.3 μ L, 53 μ mol), the mixture was heated to 60 °C for 18 h. The solvent was removed and the crude product subjected to silica gel chromatography with CH_2Cl_2 . The combined product eluents were dried *in vacuo* and the complex **5b** was obtained as an orange solid (18 mg, 70%). ¹H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.92 (d, J = 5.2 Hz, 1H), 8.83 (s, 1H), 8.65 (dd, J = 7.6, 1.2 Hz, 1H), 8.48 (dd, J = 8.4, 1.2 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.81 (td, J = 8.4, 1.6 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 7.55–7.57 (m, 2H), 7.28–7.32 (m, 2H), 7.20–7.24 (m, 1H), 7.00 (td, J = 6.6, 1.2 Hz, 1H), 5.44 (s, 2H), 5.08 (s, 5H). ¹³C-NMR (100 MHz, $CDCl_3$): δ (ppm) 202.6, 199.8, 167.2, 165.4, 164.4, 157.1, 144.1, 142.8, 142.5, 137.8, 137.0, 131.9, 128.7, 128.4, 127.2, 125.5, 124.6, 122.9, 120.6, 119.9, 117.3, 84.5, 43.4. IR (film): ν (cm^{-1}) 2925,

2854, 1930, 1690, 1649, 1581, 1561, 1474, 1390, 1319, 1229, 1180, 779, 731, 701. HRMS calcd for $C_{30}H_{21}N_2O_3Ru$ ($M+H$)⁺ 559.0598, found 559.0608.

Complex 6

Method a: 3-(2-Pyridyl)-1,8-naphthalimide (**1a**) (70 mg, 255 μ mol) and $[Ru(MeCN)_3([9]aneS_3)](CF_3SO_3)_2$ (269 mg, 383 μ mol) were dissolved in 5 mL DMF, triethylamine (92 μ L, 663 μ mol) was added, and the reaction mixture was heated to 90 °C for 18 h. The solution was cooled to ambient temperature, the solvent was removed, and the crude product subjected to silica gel chromatography with first acetonitrile followed by acetonitrile/water/sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of acetonitrile-water, and NH_4PF_6 was added. The solution was centrifuged, the resulting orange filter cake washed three times with water, and dried *in vacuo* to provide the monoacetonitrile **6** complex as orange solid (145 mg, 77%).

Method b: Tetraacetonitrile complex **10** (15 mg, 22 μ mol) was dissolved in 3 mL DMF, 1,4,7-trithiacyclonane was added, and the mixture heated to 90 °C for 24 h. The solution was cooled to ambient temperature, the solvent was removed, and the crude product subjected to silica gel chromatography with first acetonitrile followed by acetonitrile/water/sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of acetonitrile-water, and NH_4PF_6 was added. The solution was centrifuged, the resulting orange filter cake washed three times with water, and dried *in vacuo* to provide monoacetonitrile complex **6** as an orange solid (12 mg, 73%). ¹H-NMR (300 MHz, CD_3CN): δ (ppm) 9.39 (s, 1H), 8.95 (d, J = 8.4 Hz, 1H), 8.86 (s, 1H), 8.77 (d, J = 5.7 Hz, 1H), 8.55 (d, J = 7.2 Hz, 1H), 8.37 (d, J = 6.9 Hz, 1H), 8.08 (t, J = 7.5 Hz, 1H), 7.85 (t, J = 7.8 Hz, 1H), 7.38 (t, J = 6.9 Hz, 1H), 3.15–3.31 (m, 2H), 2.76–3.00 (m, 6H), 2.08–2.46 (m, 7H). ¹³C-NMR (75 MHz, CD_3CN): δ (ppm) 194.4, 189.9, 165.4, 164.5, 163.9, 153.1, 144.9, 141.5, 139.4, 139.0, 130.8, 129.5, 126.7, 124.0, 123.7, 123.6, 121.7, 119.4, 37.0, 36.5, 34.5, 33.6, 31.8, 30.6. IR (film): ν (cm^{-1}) 2991, 1974, 1675, 1582, 1564, 1476, 1412, 1386, 1322, 1248, 837, 778, 748. HRMS calcd for $C_{25}H_{24}N_3O_2RuS_3$ ($M-PF_6$)⁺ 596.0069, found 596.0078.

Complex 7

Monoacetonitrile complex **6** (15 mg, 20 μ mol) was dissolved in 4 mL DMF and purged with CO gas for 30 s. The solution was heated to 95 °C for 3 h. The solvent was removed and the crude product subjected to silica gel chromatography with first acetonitrile followed by acetonitrile/water/sat. KNO_3 solution 50:3:1. The combined product eluents were dried *in vacuo*, dissolved in a minimal amount of acetonitrile-water, and NH_4PF_6 was added. The solution was centrifuged, the resulting yellow filter cake washed three times with water, and dried *in vacuo* to provide monoacetonitrile complex **7** as a yellow solid (13 mg, 87%). ¹H-NMR (400 MHz, CD_3CN): δ (ppm) 9.36 (s, 1H), 8.95 (dd, J = 8.4, 1.2 Hz, 1H), 8.86 (s, 1H), 8.77 (dt, J = 5.2, 0.8 Hz, 1H), 8.55 (dd, J = 7.2, 1.2 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.08 (td, J = 8.4, 0.8 Hz, 1H), 7.85 (dd, J = 8.4, 7.2 Hz, 1H), 7.38 (td, J = 5.6, 1.2 Hz, 1H), 3.17–3.30 (m, 2H), 2.86–3.00 (m, 4H), 2.77–2.85 (m, 2H), 2.28–2.46 (m, 3H), 2.19–2.25 (m, 1H). ¹³C-NMR (100 MHz, CD_3CN): δ (ppm) 194.4, 189.9, 165.4, 164.5, 163.9, 153.1, 144.9, 141.5, 139.4, 138.9, 130.8, 129.4, 126.7, 124.0, 123.7, 123.6, 121.7, 119.4, 37.0, 36.5, 34.5, 33.6, 31.8, 30.6. IR (film): ν (cm^{-1}) 3164, 3053, 2839, 1991, 1675, 1584, 1474, 1387, 1317, 1266, 834, 780, 745. HRMS calcd for $C_{24}H_{21}N_2O_3RuS_3$ ($M-PF_6$)⁺ 582.9752, found 582.9752.

Complex 8

Monoacetonitrile complex **6** (16 mg, 22 μ mol) was dissolved in 2 mL DMF, NaSCN (3 mg, 32 μ mol) in water (100 μ L) was added, and the solution was heated to 95 °C for 12 h. The

solvent was removed and the crude product subjected to silica gel chromatography with CH₂Cl₂-MeOH (gradient 20:1 to 10:1). The combined product eluents were dried *in vacuo* and the thiocyanate complex **8** was obtained as a red solid (3 mg, 23%). The ruthenium coordination of the ambident NCS ligand through nitrogen was verified by ¹⁵N-NMR and a crystal structure of a benzylated derivative (see Supporting Information). ¹H-NMR (300 MHz, DMSO-*d*₆): δ (ppm) 11.42 (s, 1H), 9.22 (dd, *J* = 8.4, 1.1 Hz, 1H), 8.96 (d, *J* = 4.8 Hz, 1H), 8.64 (s, 1H), 8.40 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 1H), 7.90 (dt, *J* = 8.4, 1.5 Hz, 1H), 7.74 (dd, *J* = 7.6, 6.6 Hz, 1H), 7.31–7.36 (m, 1H), 3.14–3.20 (m, 1H), 2.87–2.97 (m, 2H), 2.64–2.76 (m, 5H), 2.07–2.19 (m, 1H), 1.83–2.01 (m, 3H). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ (ppm) 215.2, 165.6, 165.4, 164.6, 152.6, 145.2, 143.1, 140.4, 137.0, 130.3, 129.1, 125.2, 124.3, 123.4, 122.8, 122.3, 119.9, 115.3, 36.6, 35.5, 35.2, 31.5, 30.3, 29.9. IR (film): ν (cm⁻¹) 3159, 3002, 2920, 2854, 2096, 1673, 1574, 1379, 1312, 1248, 1016, 951, 841, 768, 739. HRMS calcd for C₂₄H₂₁N₃O₂RuS₄Na (M+Na)⁺ 635.9453, found 635.9467.

Complex 9

Monoacetonitrile complex **6** (16 mg, 22 μmol) was dissolved in 2 mL DMF, KSeCN (5 mg, 32 μmol) in water (100 μL) was added, and the solution was heated to 95 °C for 12 h. The solvent was removed and the crude product subjected to silica gel chromatography with CH₂Cl₂-MeOH (gradient 20:1 to 10:1). The combined product eluents were dried *in vacuo* and the selenocyanate complex **9** was obtained as a red solid (4 mg, 30%). The ruthenium coordination of the ambident SeCN ligand through selenium was verified by a crystal structure of a benzylated derivative (see Supporting Information). ¹H-NMR (500 MHz, DMSO-*d*₆): δ (ppm) 11.39 (s, 1H), 9.22 (dd, *J* = 8.0, 1.0 Hz, 1H), 8.94–8.95 (m, 1H), 8.64 (s, 1H), 8.39 (dd, *J* = 7.0, 1.0 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 7.90 (ddd, *J* = 8.5, 7.5, 1.5 Hz, 1H), 7.73 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.32 (ddd, *J* = 7.0, 5.5, 1.5 Hz, 1H), 3.15–3.19 (m, 1H), 2.90–2.98 (m, 2H), 2.64–2.77 (m, 4H), 2.50–2.54 (m, 1H), 2.06–2.13 (m, 1H), 1.87–1.99 (m, 3H). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ (ppm) 165.6, 165.3, 164.4, 152.5, 145.4, 143.2, 140.7, 137.0, 136.9, 130.4, 129.4, 125.0, 123.5, 122.8, 122.3, 119.9, 115.3, 108.4, 36.5, 36.3, 35.3, 31.3, 31.1, 30.0. IR (film) ν (cm⁻¹) 3344, 3224, 3003, 2921, 2851, 2098, 1674, 1575, 1507, 1432, 1406, 1378, 1321, 1256, 1019, 827, 778. HRMS calcd for C₂₄H₂₁N₃O₂RuS₃SeNa (M+Na)⁺ 683.8904, found 683.8903.

Complex 10

3-(2-Pyridyl)-1,8-naphthalimide (**1a**) (78 mg, 0.28 mmol), [RuCl₂(η⁶-C₆H₆)]₂ (71 mg, 0.14 mmol), KPF₆ (199 mg, 1.08 mmol), and K₂CO₃ (110 mg, 0.80 mmol) were dissolved in acetonitrile and heated to reflux for 16 h. The solvent was removed and the crude product subjected to silica gel chromatography with CH₂Cl₂-MeOH (gradient 35:1 to 20:1). The combined product eluents were dried *in vacuo* and the tetraacetonitrile complex **10** was obtained as a red solid (77 mg, 40%). ¹H-NMR (300 MHz, CD₃CN): δ (ppm) 9.21 (br s, 1H), 9.14 (dd, *J* = 8.4, 1.2 Hz, 1H), 9.04–9.06 (m, 1H), 8.65 (s, 1H), 8.52 (dd, *J* = 7.2, 1.2 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.85–7.91 (m, 1H), 7.78 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.28 (ddd, *J* = 7.2, 5.7 Hz, 1.2 Hz, 1H), 2.55 (s, 3H), 2.14 (s, 6H), 1.99 (s, 3H). ¹³C-NMR (125 MHz, CD₃CN): δ (ppm) 215.8, 167.2, 165.0, 164.1, 152.7, 145.8, 143.4, 140.6, 136.8, 130.3, 128.8, 126.2, 124.8, 123.4, 122.2, 121.7, 121.5, 118.9, 115.6, 2.8. IR (film): ν (cm⁻¹) 3363, 2924, 2851, 2271, 1681, 1578, 1561, 1473, 1316, 1242, 830, 776, 757, 557. HRMS calcd for C₂₃H₁₈N₅O₂Ru (M-CH₃CN-PF₆)⁺ 498.0505, found 498.0487.

Protein kinase assays

The protein kinase selectivity profile of complex **7** at an assay concentration of 10 μM was derived from an active-site-directed affinity screening against 442 human protein kinases (KINOMEscan, DiscoveRx). See Supporting Information for additional data. Determination

of IC₅₀-values: Various concentrations of the ruthenium complexes or the ligand **1a** were incubated at room temperature in 20 mM MOPS, 30 mM Mg(OAc)₂, 0.8 µg/µL BSA, 5% DMSO (**7–9**, **7Me**) or 10% DMSO (**5a** and **1a**) (resulting from the inhibitor stock solution), pH 7.0, in the presence of CaCl₂, (500 µM), calmodulin (1 µM), substrate ZIPTide (62.5 µM), and human MYLK (purchased from Millipore as MLCK) (6.9 nM). After 30 min, the reaction was initiated by adding ATP to a final concentration of 100 µM and approximately 0.1 µCi/µL [^γ-³³P] ATP. Reactions were performed in a total volume of 25 µL. After 45 min, the reaction was terminated by spotting 15 µL on a circular P81 phosphocellulose paper (2.1 cm diameter, Whatman), followed by washing three times with 0.75% phosphoric acid and once with acetone. The dried P81 papers were transferred to a scintillation vial, 5 mL of scintillation cocktail was added, the counts per minute (CPM) were measured with a Beckmann Coulter™ LS6500 Multi-Purpose Scintillation Counter, and the IC₅₀ values were defined to be the concentration of inhibitor at which the counts per minute (CPM) were 50% of the control sample, corrected by the background CPM.

Cell viability assays

Human melanoma cell lines (451Lu, WM3918) were isolated as previously described²⁹ and were further cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum. Normal human primary melanocytes (FOM102010) and fibroblasts (FF2508) were isolated from the human epidermis of neonatal foreskins and cultured as described.^{30,31} Cells (5000/well) were seeded in 96-well plates and allowed to adhere overnight. The following day, complex **5a**, **7**, or DMSO vehicle control were added to the cells at different concentrations and the cells were incubated for another 96 h. Cells were then directly incubated with MTS substrate (CellTiter-96 Aqueous One Solution Cell Proliferation Assay, Promega).²⁴ Absorbance was measured at 490 nm as per the supplier's instructions and percent proliferation was normalized to the absorbance of DMSO-treated cells. For each cell line and treatment, the absorbance values of at least 4 wells were used to analyze the data.

Single-crystal X-ray diffraction studies

The intensity data sets for all compounds were collected at 100 K using a STOE IPDS2 (**5b**) or IPDS-2T (**7**, **10**) system. The data were corrected for absorption effects using multi scanned reflections (**5b**)³² or indexed faces (**7**, **10**),³³ respectively. The structures were solved using direct methods (SIR-92³⁴ for **10** and SIR2008³⁵ for **5b**, **7**) and refined using the full matrix least squares procedure implemented in SHELX-97.³⁶ Hydrogen atoms were included at calculated positions, N bonded H atoms in **10** were located and refined isotropically. The crystal of **5b** was twinned. Only the reflections of one twin domain were used for refinement. In **5b** two positions of a disordered CH₂Cl₂ molecule were refined with a total occupation of 0.65 in the asymmetric unit. In **7** one hexafluorophosphate anion was disordered. High displacement factors indicate non resolved disorder of the solvent ether molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the National Institutes of Health USA (CA114046) for support of this research. S.B. acknowledges a stipend from the Fonds der Chemischen Industrie.

References

1. For organometallics in biological and medicinal chemistry, see: (a) Severin K, Bergs R, Beck W. *Angew. Chem. Int. Ed.* 1998; 37:1634–1654. (b) Jaouen G. *J. Organomet. Chem.* 1999; 589:1–126. (c) Metzler-Nolte N. *Angew. Chem. Int. Ed.* 2001; 40:1040–1043. (d) Fish RH, Jaouen G. *Organometallics*. 2003; 22:2166–2177. (e) Schatzschneider U, Metzler-Nolte N. *Angew. Chem. Int. Ed.* 2006; 45:1504–1507. (f) Alberto R. *J. Organomet. Chem.* 2007; 692:1179–1186. (g) Hartinger CG, Dyson PJ. *Chem. Soc. Rev.* 2009; 38:391–401. [PubMed: 19169456] (h) Fish RH. *Austr. J. Chem.* 2010; 63:1505–1513. (i) Hillard EA, Jaouen G. *Organometallics*. 2011; 30:20–27. (j) Gasser G, Ott I, Metzler-Nolte N. *J. Med. Chem.* 2011; 54:3–25.
2. Haas KL, Franz KJ. *Chem. Rev.* 2009; 109:4921–4960. [PubMed: 19715312]
3. (a) Debreczeni JÉ, Bullock AN, Atilla GE, Williams DS, Bregman H, Knapp S, Meggers E. *Angew. Chem. Int. Ed.* 2006; 45:1580. (b) Maksimoska J, Feng L, Harms K, Yi C, Kissil J, Marmorstein R, Meggers E. *J. Am. Chem. Soc.* 2008; 130:15764–15765. [PubMed: 18973295]
4. Williams DS, Carroll PJ, Meggers E. *Inorg. Chem.* 2007; 46:2944–2946. [PubMed: 17375909]
5. Maksimoska J, Williams DS, Atilla-Gokcumen GE, Smalley KSM, Carroll PJ, Webster RD, Filippakopoulos P, Knapp S, Herlyn M, Meggers E. *Chem. Eur. J.* 2008; 14:4816–4822.
6. Wilbuer A, Vlecken DH, Schmitz DJ, Kräling K, Harms K, Bagowski CP, Meggers E. *Angew. Chem. Int. Ed.* 2010; 49:3839–3842.
7. For work from other groups on the design of metal-containing protein kinase inhibitors, see: (a) Spencer J, Mendham AP, Kotha AK, Richardson SCW, Hillard EA, Jaouen G, Male L, Hursthouse MB. *Dalton Trans.* 2009:918–921. [PubMed: 19173072] (b) Biersack B, Zoldakova M, Effenberger K, Schobert R. *Eur. J. Med. Chem.* 2010; 45:1972–1975. [PubMed: 20149940]
8. Feng L, Geisselbrecht Y, Blanck S, Wilbuer A, Atilla-Gokcumen GE, Filippakopoulos P, Kräling K, Celik MA, Harms K, Maksimoska J, Marmorstein R, Frenking G, Knapp S, Essen L-O, Meggers E. *J. Am. Chem. Soc.* 2011; 133:5976–5986. [PubMed: 21446733]
9. Meggers E, Atilla-Gokcumen GE, Bregman H, Maksimoska J, Mulcahy SP, Pagano N, Williams DS. *Synlett.* 2007; 8:1177–1189.
10. (a) Bregman H, Williams DS, Meggers E. *Synthesis*. 2005:1521–1527. (b) Pagano N, Maksimoska J, Bregman H, Williams DS, Webster RD, Xue F, Meggers E. *Org. Biomol. Chem.* 2007; 5:1218–1227. [PubMed: 17406720]
11. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. *Science*. 2002; 298:1912–1934. [PubMed: 12471243]
12. Gill TP, Mann KR. *Organometallics*. 1982; 1:485–488.
13. This should be in analogy to transition metal-perfluoroalkyl complexes which typically possess stronger M-C bonds compared to their nonfluorinated counterparts. See, for example: Elschenbroich C. *Organometallics* (3rd Ed.). 2006 Weinheim Wiley-VCH
14. Landgrafe C, Sheldrick WS. *Dalton Trans.* 1994:1885–1893.
15. See, for example: (a) Ryabov AD, Sukharev VS, Alexandrova L, Le Lagadec R, Pfeffer M. *Inorg. Chem.* 2001; 40:6529–6532. [PubMed: 11720516] (b) Bregman H, Carroll PJ, Meggers E. *J. Am. Chem. Soc.* 2006; 128:877–884. [PubMed: 16417378] (c) Mulcahy SP, Gründler K, Frias C, Wagner L, Prokop A, Meggers E. *Dalton Trans.* 2010; 39:8177–8182. [PubMed: 20689887]
16. For analogous reactions, see: (a) Fernandez S, Pfeffer M, Ritleng V, Sirlin C. *Organometallics*. 1999; 18:2390–2394. (b) Leyva L, Sirlin C, Rubio L, Franco C, Le Lagadec R, Spencer J, Bischoff P, Gaidon C, Loeffler J-P, Pfeffer M. *Eur. J. Inorg. Chem.* 2007:3055–3066. (c) Djukic J-P, Sortais J-B, Barloy L, Pfeffer M. *Eur. J. Inorg. Chem.* 2009:817–853.
17. Fabian MA, et al. *Nat. Biotechnol.* 2005; 23:329–336. [PubMed: 15711537]
18. Karaman MW, et al. *Nat. Biotechnol.* 2008; 26:127–132. [PubMed: 18183025]
19. Katoh K, Kano Y, Ookawara S. *Trends Cell Mol. Biol.* 2005; 1:83–88.
20. Cheng Y-C, Prusoff WH. *Biochem. Pharmacol.* 1973; 22:3099–3108. [PubMed: 4202581]
21. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S. *Nature*. 1997; 389:990–994. [PubMed: 9353125]
22. Bollag G, et al. *Nature*. 2010; 467:596–599. [PubMed: 20823850]

23. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo J, Nolop K, Chapman PB. *New. Engl. J. Med.* 2010; 363:809–819. [PubMed: 20818844]
24. Berg K, Zhai L, Chen M, Kharazmi A, Owen TC. *Parasitol. Res.* 1994; 80:235–239. [PubMed: 8036237]
25. Pinto-Bazurco Mendieta MAE, Negri M, Jagusch C, Hille UE, Müller-Vieira U, Schmidt D, Hansen K, Hartmann RW. *Bioorg. Med. Chem. Lett.* 2008; 18:267–273. [PubMed: 18024111]
26. Brotschi C, Mathis G, Leumann CJ. *Chem. Eur. J.* 2005; 11:1911–1923.
27. Ranger M, Rondeau D, Leclerc M. *Macromolecules.* 1997; 30:7686–7691.
28. Kündig EP, Monnier FR. *Adv. Synth. Catal.* 2004; 346:901–904.
29. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M. *Cancer Res.* 2005; 65:9328–9337. [PubMed: 16230395]
30. Hsu M-Y, Shih D-T, Meier FE, Van Belle P, Hsu J-Y, Elder DE, Buck CA, Herlyn M. *Am. J. Pathol.* 1998; 153:1435–1442. [PubMed: 9811334]
31. Fukunaga-Kalabis M, Martinez G, Liu ZJ, Kalabis J, Mrass P, Weninger W, Firth SM, Planque N, Perbal B, Herlyn M. *J. Cell Biol.* 2006; 175:563–569. [PubMed: 17101694]
32. Spek, AL. *PLATON, A Multipurpose Crystallographic Tool.* The Netherlands: Utrecht University, Utrecht; 1998.
33. X-Area, Version 1.54. STOE & Cie GmbH; 2009.
34. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A. *J. Appl. Cryst.* 1993; 26:343–350.
35. Burla MC, Caliandro R, Camalli M, Carrozzini B, Cascarano GL, De Caro L, Giacovazzo C, Polidori G, Siliqi D, Spagna R. *J. Appl. Cryst.* 2007; 40:609–613.
36. Sheldrick GM. *Acta Cryst. A.* 2008; 64:112–122. [PubMed: 18156677]

Figure 1. Previous and new design for metal complexes as protein kinase inhibitors. Shown are the intended interactions of the so-called “pharmacophore chelate ligand” with the hinge region of the ATP-binding site of protein kinases.

Figure 2.

Crystal structure of half-sandwich complex **5b**. A disordered CH₂Cl₂ solvent molecule is omitted. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å) and angles (°): Ru1-C1 = 2.073(8), Ru1-N15 = 2.094(9), Ru1-C27 = 2.230(10), Ru1-C30 = 2.274(7), Ru1-C111 = 1.838(12), C1-Ru-N15 = 78.8(4), C111-Ru1-C1 = 87.2(4), C111-Ru1-N15 = 97.0(4).

Figure 3.

Crystal structure of the 1,4,7-trithiacyclononane complex **7**. Solvent molecules (MeCN and diethyl ether) and the hexafluorophosphate counterions are omitted for clarity. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å) and angles (°): Ru1-C1 = 2.075(4), Ru1-N15 = 2.095(4), Ru1-C26 = 1.864(5), Ru1-S1 = 2.3419(11), Ru1-S2 = 2.4169(11), Ru1-S3 = 2.4077(12), C1-Ru1-N15 = 78.72(15), C1-Ru1-S3 = 87.53(12), C1-Ru1-C26 = 92.29(17), N15-Ru1-S3 = 84.01(10), N15-Ru1-C26 = 94.91(16).

Figure 4.

Crystal structure of the tetraacetonitrile precursor complex **10**. ORTEP drawing with 50% probability thermal ellipsoids. Selected bond distances (Å) and angles (°): Ru1-C1 = 2.041(4), Ru1-N14 = 2.043(4), Ru1-N100 = 2.018(4), Ru1-N200 = 2.165(4), Ru1-N300 = 2.049(4), Ru1-N400 = 2.018(4), C1-Ru1-N14 = 80.35(15), C1-Ru1-N100 = 92.06(15), C1-Ru1-N200 = 175.11(16), C1-Ru1-N300 = 102.31(15), C1-Ru1-N400 = 90.49(15), C100-N100-Ru1 = 179.4(4), C200-N200-Ru1 = 169.0(4), C300-N300-Ru1 = 168.4(3), C400-N400-Ru1 = 174.5(3).

Figure 5. Protein kinase selectivity of complex **7** as determined by an active-site-directed affinity screening (KINOMEscan, DiscoverX) against 442 human protein kinases. Representation of the main hits (< 0.1% of control; 0% = highest affinity, 100% = no affinity) within the human kinase dendrogram which displays the protein kinase families and the evolutionary relationships between the individual kinases. See Supporting Information for additional data.

Figure 6.

IC₅₀ curves of complex **7** and the methylated control complex **7Me** against MYLK at ATP concentrations of 100 μM. The pyridylnaphthalimide ligand **1a** does not show any inhibition of MYLK even at a concentration of 1 mM.

Figure 7. Effects of the pyridyl-naphthalimide complexes **7** and **5a** on the proliferation of human-derived cells. Cellular proliferation of melanoma cell lines (451Lu and WM3918), human melanocytes (FOM102010), and human fibroblasts (FF2508) were assessed with the MTS assay. Data show the mean value of three independent experiments including the standard deviations. Proliferation is given as % of DMSO control experiments.

Scheme 1.
 Synthesis of 3-(2-pyridyl)-1,8-naphthalimide (**1a**) and *N*-benzyl-3-(2-pyridyl)-1,8-naphthalimide (**1b**).

Scheme 2.

Synthesis of ruthenium half-sandwich complexes **5a** and **5b** as racemic mixtures (only one enantiomer shown).

Scheme 3.

Synthesis of the octahedral 1,4,7-trithiacyclononane ruthenium complexes **7–9** by two different routes. Only one enantiomer of the formed racemic mixtures **6–9** is shown.

Table 1Crystallographic data for ruthenium complexes **5b**, **7**, and **10**^a

	5b·1.3(CH₂Cl₂)	7·Et₂O·CH₃CN	10
formula	C ₃₀ H ₂₀ N ₂ O ₃ Ru, 0.65(CH ₂ Cl ₂)	[C ₂₄ H ₂₁ N ₂ O ₃ RuS ₂] ⁺ , PF ₆ [−] , Et ₂ O, CH ₃ CN	[C ₂₅ H ₂₁ N ₆ O ₂ Ru] ⁺ , PF ₆ [−]
fw	612.88	883.88	683.52
a(Å)	23.7464(13)	12.6950(4)	8.3493(6)
b(Å)	7.0753(5)	14.1048(4)	11.6436(8)
c(Å)	29.415(2)	21.4127(7)	14.5174(8)
α(°)		102.734(2)	102.461(5)
β(°)		95.437(3)	101.109(5)
γ(°)		99.004(3)	101.134(5)
V(Å ³)	4942.2(6)	3660.4(2)	1311.06(15)
Z	8	4	2
space group	Pbca	P I	P I
d _{calcd} (Mg/m ³)	1.647	1.604	1.731
μ(mm ^{−1})	0.814	0.716	0.739
θ range(°)	1.38 – 25	4.65 – 25	4.71 – 25
no. of indep. reflections	3082	12812	4476
no. of parameters	381	960	391
wR2 (all data) ^b	0.121	0.136	0.059
R1 (I > 2σ(I)) ^b	0.053	0.051	0.040
CCDC no. ^c	812716	812717	812718

^a MoKα radiation (λ = 0.71073 Å).^b R1 = Σ||F_o| − |F_c||/Σ|F_o|; wR2 = [w(F_o² − F_c²)/Σw(F_o²)]^{1/2}.^c Crystallographic data (excluding structure factors) have been deposited in the Cambridge Crystallographic Data Center. CIF files can be obtained from the CCDC free of charge via http://www.ccdc.cam.ac.uk/data_request/cif.